Pathogenic Fungi Associated with Sand Pine Root Disease in Florida

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ABSTRACT

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Eight known or suspected pathogenic fungi were isolated from roots of diseased sand pine (*Pinus clausa*) in Florida. *Phytophthora cinnamomi* was the pathogen most frequently isolated from planted trees, but it was not isolated from trees in natural stands. *Inonotus circinatus* was the pathogen most frequently isolated from trees 20 yr or older. *Armillariella tabescens* and *Verticicladiella procera* were isolated frequently from trees of most ages in both planted and natural stands, but they did not appear to be primary etiological factors. *Heterobasidion annosum*, *Macrophomina phaseolina*, and *Phaeolus schweinitzii* were isolated infrequently. *Phytophthora parasitica* was isolated from nursery seedling roots on only three occasions.

Sand pine (*Pinus clausa* (Chapm.) Vasey) is a relatively small, short-lived southern yellow pine native to Florida's sand hills. Botanists and foresters generally recognize two distinct varieties, Choctawhatchee (*P. clausa* var. *immuginata* Ward), native to Florida's western panhandle, and Ocala (*P. clausa* var. *clausa* Ward), native to peninsular Florida. Interest in commercial management of sand pine has increased in recent years because of its ability to grow well on deep, infertile, acid, droughty sands—sites on which other commercially important tree species develop poorly if at all. About 810,000 ha in Florida are classified as sand hills. Commercial forest nurseries in the state currently produce more than 10 million sand pine seedlings annually to meet sandhill regeneration needs.

Mushroom root rot, caused by *Armillariella tabescens* (Scop. ex Fr.) Singer (=*Clitocybe tabescens* Bres.), has been considered the most important disease limiting successful management of sand pine, especially the Ocala variety (1,17). Our recent field observations, however, indicated that the etiology of sand pine root disease is complex. This paper summarizes diagnostic studies that included a statewide survey (5) of diseased sand pines in seed orchards, forest nurseries, plantations, and natural stands. A preliminary report has been published (6).

MATERIALS AND METHODS

Diseased trees in about 40 stands of sand pine within the natural and commercial ranges of the species in northern and central Florida were examined. Affected trees displayed varying degrees and combinations of crown thinning and/or foliar discoloration, basal cankers ("cat faces"), basal

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resinosis, and wind-throw or leaning (2,3,16). Stands varied in age from about 3 to more than 50 yr. Within these stands, soil/site relationships were evaluated by examining soil textures and profiles in each of three soil cores taken with a bucket auger to a depth of 1.8 m. First-year seedlings in seven commercial forest nurseries and three ornamental nurseries were also examined.

Roots were excavated and examined in the field. Samples representing the range of symptom types encountered were transported on ice to the laboratory. Portions of necrotic root systems of nursery seedlings and feeder roots of larger trees were plated directly onto a medium selective for Oomycetes (PARP) (10). Wood chips from taproots, lateral roots, and the root collar area, representing each observed symptom pattern (decayed, stained, resin-soaked, etc.), were plated onto malt-extract agar (MEA) (15 g malt extract + 15 g agar + 1,000 ml deionized H_2O) and/or a medium selective for Basidiomycetes (OPP) (20 g malt extract + 17 g agar + 100 mg streptomycin sulfate + 1 ml 50% lactic acid + 2.5 ml of a stock solution of 0.48 g orthophenylphenol in 20 ml 95% ethanol + 1,000 ml deionized H_2O). Sample size not precluding, five to 10 isolations were attempted onto one or both of the media for each symptom type within each root category (taproot, lateral root, etc.) from each tree sampled. Half of the wood chips from which isolations were attempted were surface-sterilized by dipping in 95% ethanol and flaming and half were plated without surface-sterilization after being carefully removed from the interior of sample roots. Plates containing PARP were examined for oomycetous pathogens after 3 days of incubation at room temperature. Plates containing MEA and OPP were examined for other root pathogens after an incubation period extending in some cases to 1 mo.

RESULTS

Eight known and/or suspected root-pathogenic fungi were isolated from roots of diseased trees. *Phytophthora cinnamomi* Rands, *Inonotus circinatus* (Fr.) Gilbertson (= *Polyporus tomentosus* Fr. var. *circinatus* Sartory & Maire), *Armillariella tabescens*, and *Verticicladiella procera* Kendrick were isolated frequently across a wide geographic area (Fig. 1). *Macrophomina phaseolina* (Tassi) Goid. was isolated with less frequency and typically from nursery seedlings or plantation trees ≤4 yr old. *Phaeolus schweinitzii* (Fr.) Pat. was isolated primarily from overmature trees of the Ocala variety in central Florida. *Heterobasidion annosum* (Fr.) Bref. and *Phytophthora parasitica* Dastur were isolated infrequently. *P. parasitica* was isolated only from roots of dying sand pine seedlings in one forest nursery that produces bare-root seedlings

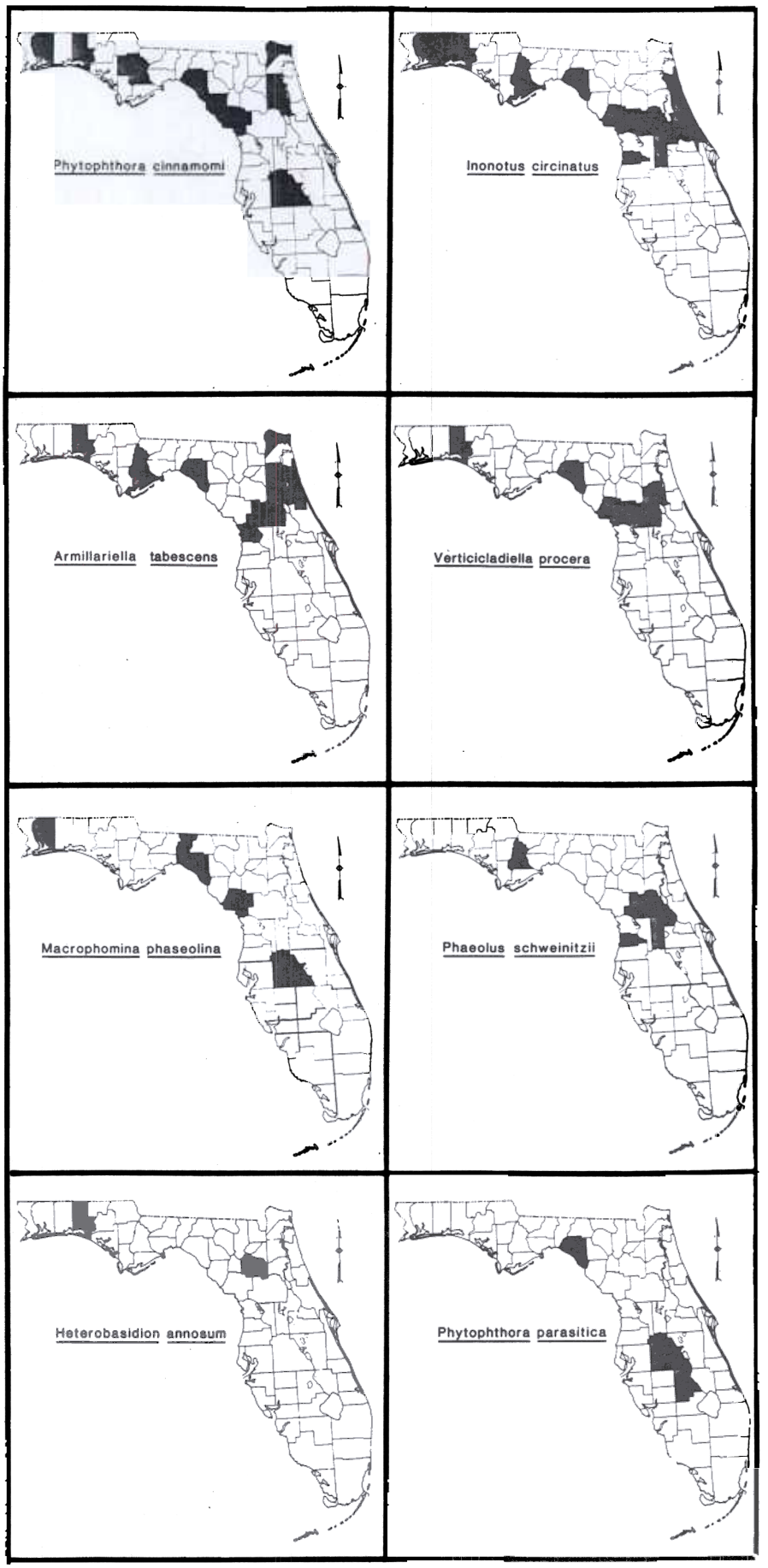

Fig. 1. Distribution by counties of known and/or suspected root-pathogenic fungi on sand pine Florida.

and two ornamental nurseries that produce containerized planting stock. Table 1 summarizes root types and conditions from which the various fungi were isolated.

Patterns of occurrence of the various fungi were not apparent with respect to variety of host (Choctawhatchee vs. Ocala) but were evident with respect to stand type and stand age (Figs. 2 and 3). For example, *P. cinnamomi* was most common in young, planted stands (≤15 yr old) and *I. circinatus* predominated in older stands (≥15 yr old). *P. cinnamomi* was not isolated from trees in natural stands (including stands established by direct seeding). Two or more fungi were commonly isolated from the same tree.

In the field, basidiocarps of *I. circinatus* and *P. schweinitzii* were often observed at or near the bases of infected mature or overmature trees (Fig. 4). Those of *I. circinatus* were commonly produced during autumn and winter, and those of *P. schweinitzii*, during mid-summer to late summer. Basidiocarps of *H. annosum* were observed beneath the duff layer at the root collars of infected trees in one thinned plantation. Although not consistently present, perforated mycelial felts beneath the bark of roots and occasionally root collars were the most reliable field signs of infections by *A. tabescens* (Fig. 4). Basidiocarps of *A. tabescens* were not observed in association with diseased sand pines. Microsclerotia of *M. phaseolina* could sometimes be detected beneath the bark of infected roots where this organism occurred (Fig. 4).

Table 1. Common types and conditions of sand pine roots from which known and/or suspected root-pathogenic fungi were isolated in Florida[a]

	Fungus[b]							
	At	Ha	Ic	Mp	Ps	Pc	Pp	Vp
Type								
Taproot		+	+	+	+			
Lateral root		+	+	+	+			
Feeder root				+				
Root collar			+					
Symptoms/conditions of root tissue								
Asymptomatic								
Resin-soaked					+			
Water-soaked								
Necrotic								
Red-brown stained								
White pocket rot								
Dry sound wood								
Stringy white rot								
Light brown rot					+			

[a] + = Positive isolation; * = most common condition of tissues yielding organism noted.
[b] At = *Armillariella tabescens*, Ha = *Heterobasidion annosum*, Ic = *Inonotus circinatus*, Mp = *Macrophomina phaseolina*, Ps = *Phaeolus schweinitzii*, Pc = *Phytophthora cinnamomi*, Pp = *Phytophthora parasitica*, and Vp = *Verticicladiella procera*.

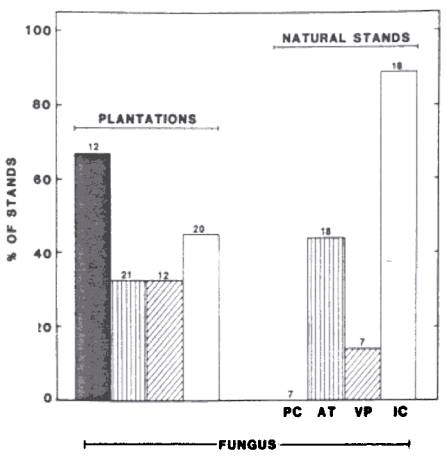

Fig. 2. Percentage of sand pine stands from which various known and/or suspected root-pathogenic fungi were isolated in Florida. PC = *Phytophthora cinnamomi*, AT = *Armillariella tabescens*, VP = *Verticicladiella procera*, IC = *Inonotus circinatus*. Numbers of stands on which percentages are based are noted at the top of each bar.

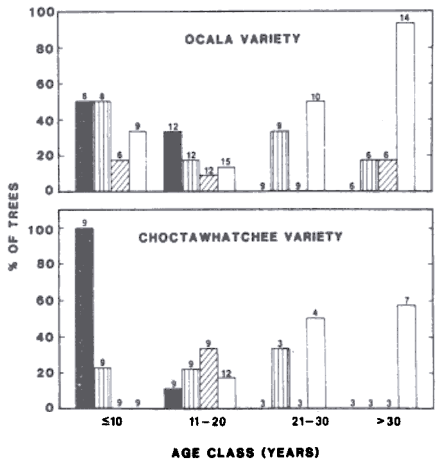

Fig. 3. Percentage of sand pine trees from which various known and/or suspected root-pathogenic fungi were isolated in Florida. ■ = *Phytophthora cinnamomi*, ▥ = *Armillariella tabescens*, ▨ = *Verticicladiella procera*, and □ = *Inonotus circinatus*. Numbers of trees on which percentages are based are noted at the top of each bar.

DISCUSSION

Root disease of sand pine in Florida is a serious problem. Losses in recent years have included mortality and quarantine of nursery seedlings (3,4), plantation failures before trees attain merchantable size, scattered mortality, and "spot kills" involving 10 or more trees of intermediate-aged (10–15 yr), mature, or overmature stands (*unpublished*). Losses sustained through disease-related growth reductions have not been quantified. To date, most mortality has occurred in the Ocala variety. Annual losses to sand pine root disease in Florida may exceed $1–2 million (5).

Further studies are needed to understand the extent to which root-infecting fungi act singly and/or in sequential or synergistic combinations in sand pine. Apparently, *I. circinatus* plays the dominant role in root disease of mature and overmature trees, whereas *P. cinnamomi* appears to be most important in young, planted trees. The infrequent occurrence of *H. annosum*, *M. phaseolina*, *Phytophthora parasitica*, and *Phaeolus schweinitzii* suggests minor roles for these pathogens in the sand pine root disease complex. These fungi could, however, become important in certain situations such as stand thinnings, overmature timber, unfavorable planting sites, or excessively wet nursery seedbeds. Webb and Dahm (23; *personal communication*) have demonstrated that *P. parasitica* is pathogenic to seedlings of both varieties of sand pine.

Based on frequency of isolation, *A. tabescens* and *V. procera* may be important in sand pine root disease. In more than 50% of the cases where these fungi were isolated, however, at least one other known or suspected root pathogen was recovered as well. In many cases, *A. tabescens* was isolated from only distal, dead, or water-soaked roots (Table 1). These roots often appeared to have been previously "cut-off" from the tree by internal resinosis, which was frequently related to infections by *P. cinnamomi* or *I. circinatus*. Rarely was *A. tabescens* isolated from resin-soaked roots, the most prominent symptom of sand pine root disease. Peterson (14) and Weaver (22) have reported a secondary and/or saprophytic role for *A. tabescens* on peach. Our observations suggest a similar role for this organism on sand pine.

Our investigations provide the first documentation of *V. procera* and *I. circinatus* on sand pine. Although not substantiated on sand pine, the roles of these organisms might be inferred from previous reports of pathogenicity on other *Pinus* spp. *V. procera* is reported to be involved in root diseases of white (*P. strobus* L.) and red (*P. resinosa* Ait.) pines in the northeastern states (12,19–21). *I. circinatus* and its close relative, *I. tomentosus* (Fr.) Gilbertson (=*Polyporus tomentosus* Fr.), are well-known root pathogens on a variety of conifers in

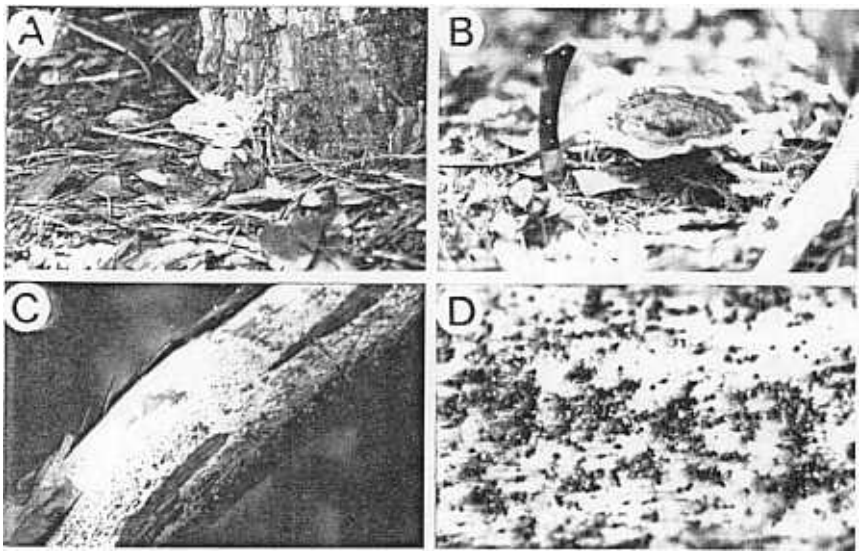

Fig. 4. Fungal signs associated with root disease of sand pine in Florida. **(A)** Fresh basidiocarp of *Inonotus circinatus* at base of tree. **(B)** Fresh basidiocarp of *Phaeolus schweinitzii* with typically zonate pileus. **(C)** Subcortical mycelial felt of *Armillariella tabescens* with characteristic perforations. **(D)** Subcortical microsclerotia of *Macrophomina phaseolina* (about ×10).

North America (13,24). In the southeastern United States, *I. circinatus* acts as a root pathogen on slash pine (*P. elliottii* Englem.) in association with basal cankers caused by fusiform rust (7–9,15). The red staining and white pocket rot we observed commonly on sand pine infected with *I. circinatus* were typical of symptoms reported for these two *Inonotus* spp. on other conifers (7,9,24).

The fact that we did not detect *P. cinnamomi* in natural stands agrees with previous data (17,18) and raises questions as to the origin of this pathogen in planted stands (plantations, seed orchards, etc.). Given that *P. cinnamomi* was introduced into the United States (25) and occurs in forest nurseries (3,11), it may have been introduced into many planting sites via infected nursery stock. Other possible modes of introduction include overland soil and water movement and spread by equipment used for harvesting, site preparation, and planting after operations in *Phytophthora*-infested areas. Recognition and regulation of these potential modes of entry, as well as the delineation of the current distribution of *P. cinnamomi* on sandhill sites, could be key elements in a preventive strategy for minimizing the future impact of sand pine root disease.

In our studies, stands for sampling were selected on the basis of the occurrence of aboveground symptoms of root disease. Accordingly, isolations were concentrated in stands with high incidence or severity of root disease. Few isolations were performed from roots of trees in natural stands ≤20 yr old. Additional isolations from young natural stands are needed to clarify aspects of the etiology and epidemiology of sand pine root disease. The scarcity of sand pine plantations ≥20 yr old accounts for the lack of data within this category (Figs. 2 and 3).

Our investigations were not designed to provide rigid conclusions regarding characteristics of sites with high hazard for sand pine root disease(s). Our observations were similar to those of others (16,18). In general, plantation sites characterized by fine-textured, poorly drained soils or those with shallow (≤1.8 m), impervious layers or evidence of poor drainage or aeration (mottling, etc.) appeared to be conducive to root disease. These are the types of soils usually regarded as favored habitat for *Phytophthora* spp. (25). In natural stands, disease/site relationships were not apparent.

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